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RESEARCH ARTICLE

Conservation tillage influences soil structure, earthworm communities and wheat root traits in a long-term organic cropping experiment

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Abstract
Background and Aims Organic farmers are showing increasing interest in using conservation tillage to improve the biological activities of soils. Here, we assessed whether conservation tillage in organic farming improves earthworm populations, root growth and soil physical quality in a sandy loam after 16 years of experiment.
Methods We compared the effect of a tillage gradient, with of two non-ploughed treatments (superficial tillage [ST] at 15 cm; very superficial tillage [VST] at 5–7 cm) and two ploughed treatments (moldboard ploughing [MP] at 30 cm; shallow moldboard ploughing [SMP] at 20 cm). Soil clod types, penetration resistance, abundance and activity of earthworms, root traits and biomass were assessed.
Results VST decreased soil compaction in topsoil (0 to 10 cm) compared to ploughed treatments (MP and SMP), but led to more compacted soil at 15 to 30 cm. Earthworm biomass (especially anecic) was higher under VST compared to MP and SMP and their galleries were better connected to the soil surface. However, there was no significant difference in the total volume of pores or diameter of galleries between 0 and 30 cm. Soil compaction in the non-ploughed treatments affected root traits, especially under VST, with lower specific root length, higher root diameter, and lower root tip elongation compared to MP and SMP.
Conclusion Biological activity did not compensate for the compaction of a sandy soil after 16 years without ploughing in organic farming. A more integrated approach (i.e. considering all 5 soil health principles) is needed to sustain soil health and functions, and meet current expectations about "ecological intensification".

Keywords Tillage gradient · Soil profile · Soil layers · Penetration resistance · Soil clods · Earthworm populations · Roots

Abbreviations

CA	Conservation agriculture
OF	Organic farming
MP	Traditional moldboard ploughing
SMP	Shallow moldboard ploughing
ST	Superficial tillage

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VST	Very superficial tillage
SRL	Specific root length
RD	Root diameter

Introduction

Globally, conservation tillage (CT) is being increasingly promoted, and involves no-till, permanent soil cover, and diversified crop rotations (Kassam et al. 2022). CT preserves topsoil fertility (Peigné et al. 2018; Soane et al. 2012), improves soil aggregate stability (Chabert and Sarthou 2020), and reduces soil degradation (such as slaking and erosion). Reducing soil tillage and stopping soil inversion (due to ploughing) minimizes the impacts on habitats of microorganisms and macro-organisms by avoiding mechanical destruction of galleries and aggregate stability. Cover cropping provides energy for food webs; thus, more macro- and microorganisms inhabit CT compared to other soil systems (Henneron et al. 2015). CT is also a technique that can increase soil C storage compared to ploughing (Young et al. 2021). However, this effect is not always confirmed when considering the soil as a whole. Most studies showed an increase in C storage in the top soil, but no effect, or even a loss of storage at greater depths (Bohoussou et al. 2022; Krauss et al. 2022).

These benefits of CT have generated interest in this practice by many farmers worldwide (Kassam et al. 2022), including organic farmers in Europe (Casagrande et al. 2015). Organic farming is based on several principles. The first of which is the principle of health with the non-use of chemically synthesized pesticides and fertilizers (IFOAM, n.d.). This translates into farming techniques such as the addition of fresh organic matter to supply plants with nutrients, and the use of mechanical tools to regulate bio-aggressors such as weeds and pests. Thus, organic farmers looking into CT practices must overcome technical and agronomical challenges to regulate weeds, burry fresh organic matter and maintain soil porosity with less or without tillage (Peigné et al. 2007). The second principle of organic farming relies on the improvement of soil organisms' abundance through more diversified crop rotations, use of organic fertilizers, and the absence of synthetic pesticides (IFOAM, n.d.). Therefore, organic farming provides a favorable ground to study how organisms can regulate pests and remediate soil structure in the context of tillage reduction (Peigné et al. 2007).

Different ways to combine OF with CT have been widely explored in the last decade (Krauss et al. 2018) mainly with agronomic trials comparing the effects of ploughed and non-ploughed techniques in OF on different parameters of soil quality and agronomic performances (weed control, crop yields). Benefits from non-ploughed techniques were observed in the first few centimeters of topsoil. In particular, C and nutrients (N, P, K) content were higher (Peigné et al. 2018), microbial activity was greater (Krauss et al. 2020), and soil density was trending lower (Peigné et al. 2018). However, crop yields tend to be lower in the non-ploughed systems of OF (Cooper et al. 2016). Several factors might contribute to this phenomenon, including competition with weeds, which is difficult to mitigate without herbicides and tillage. Increased topsoil compaction below tillage depth has also been reported for different soil types (Cooper et al. 2016) in both OF and conventional farming (Soane et al. 2012). Peigné et al. (2018) showed that the topsoil of sandy loam soil was still denser after 10 years of superficial tillage in OF compared to ploughed treatments. Krauss et al. (2018) also recorded a denser soil layer at 10 and 20 cm depth, below the tilled layer.

Without tillage, only abiotic and biotic processes prevent and/or remove soil compaction (Drewry 2006). In clay soils, successive cycles of wetting and drying of the soil (e.g., dry periods or in winter with frost) lead to the swelling and shrinking of clay, creating porous cracks. Roots and soil organisms, with a large influence from earthworms, also help to maintain and create porosity (Wendel et al. 2022). However, a significant abundance and activity of soil organisms and roots is required to maintain or increase soil porosity. Several studies showed that more soil organisms, such as earthworms are present in CT (Pelosi et al. 2014), including under OF (Kuntz et al. 2013). However, these results vary with soil cover, soil type and tillage practices (Peigné et al. 2009).

Few field-based studies have considered simultaneously earthworm communities, macropore building and root exploration. A long-term experiment by Capowiez et al. (2009) showed that more anecic earthworms were present under reduced tillage compared to ploughing. This increase was correlated with the increase of macropores at greater soil depth, compensating higher bulk density at this soil depth. However, the magnitude of root exploration changes in no-ploughed systems remains unquantified. To enhance the understanding of this phenomenon, root biomass measurements provide a clear indication of the quantity of plant belowground tissues, and provides information on plant nutrient-water acquisition capacity, interactions with soil organisms, and litter inputs to the soil. The vertical distribution of roots also provides evidence of the rooting depth, indicating potential issues with soil structure when roots fail to penetrate a given soil layer. Finally, root morphological traits (e.g., specific root length, root diameter, root tip elongation) are affected by the mechanical resistance of soil to deformation, providing useful information on soil exploration constraints and mechanical stress. In our study, root traits represent ‘response traits’ which means that their adaptation to the soil is evaluated, rather than their potential effect on it (‘effect traits’).

Thus, determining how no-till affects soil structure and biological activities (earthworms, roots) would provide an opportunity to inform scientists and farmers about practices suitable to combine tillage reduction and organic farming, where nutrient availability is especially important to maintain yields. To address this question, we benefited from a long-term experiment established in 2005, the ‘THIL’ trial, in which we compared conservation tillage and ploughing techniques under OF conditions. Previous results, after 10 years of experiment under a winter wheat crop, showed that soil fertility was better in the first 15 cm soil depth with very superficial tillage, resulting in more nutrients, C, porosity and roots (Peigné et al. 2018). However, below this depth, soil was more compact, roots became scarcer, and there was no marked effect on the abundance and activity of earthworms (assessed visually). Thus, here, we aimed to explore the results of this field experiment after 16 years. Specifically, we sought to confirm whether the results after 10 years still held at 16 years. We also conducted an in-depth analysis of earthworm activity (X-ray tomography analysis) and root growth (biomass and morphological traits) in order to assess the changes in soil biological functionalities when ploughing is stopped.

Materials and methods

Experimental design

The “Thil” trial (45° 49′ 9.44″ N and 5° 2′ 2.62″ E) was set up in 2004–5 in south-eastern France. The soil type is a calcareous fluvisol developing on a recent alluvium. The soil texture is composed of 53% sand, 32% silt, and 15% clay; therefore a sandy loam soil. The pH is 8.5. Below 60 cm, soil texture is not spatially homogeneous, due to heterogeneity in sand and gravel deposits. The climate is classified as semi-continental with Mediterranean influences. The mean annual temperature is 11.4°C, and cumulative annual rainfall was 825 mm in 2021 (20mm below the average annual rainfall for the region). The “Thil” cropping system is an irrigated system with spring crops (maize and soybean), winter wheat, and legumes as a cover crop. This system is representative of the organic stockless grain systems found in this region. Land conversion to organic farming (EU 2092/91) started in 1999. Crop rotation is based on Maize (*Zea mays* L.)- Soybean (*Glycine max L.)*- Winter wheat (*Triticum aestivum L.),* with cereal cover crops between maize and soybean, and legume cover crops between winter wheat and maize. Soybean and maize are intensively irrigated each year (around 300 mm), whereas winter wheat is irrigated according to climatic conditions (from 30 to 100 mm). Soil measurements were performed between November 2004 and March 2005 on 3-year alfalfa (*Medicago sativa*, 2002–2005), just before the beginning of the experiment (with a maize in 2005), to determine an initial point. Soil and root measurements were performed after 10 years of the experiment (Peigné et al. 2018). Soil and root measurements were performed again after 16 years of the experiment (current study) on winter wheat in 2020–2021 (at the end of the experiment, after four crop rotations).

The experimental design consisted of four tillage treatments, representing a tillage gradient, replicated randomly three times. The experimental field of 1.5 ha contained 12 experimental plots, each measuring 80×12 m^2 (length x width). The plots were separated by 2-m-wide grass strips. All plots were irrigated. The four tillage treatments were selected according to their expected effect on soil biology and soil structure, and represented a tillage depth gradient.

Specifically, we implemented two ploughed treatments (moldboard ploughing at 30 cm depth [MP] and shallow ploughing at 20 cm with no skim coulter [SMP]), and two non-ploughed treatments without soil inversion (superficial tillage at 15 cm with a chisel plough [ST] and very superficial tillage at 5–7 cm with rotary and/or chisel tools [VST]). In 2005 and 2008, direct sowing under rolled mulch was tested on VST plots (maize on rolled alfalfa in 2005 and soybean on rolled rye in 2008). The seedbed was prepared with a rotary harrow in all treatments. Weeds were mechanically destroyed by harrowing and hoeing the soil in the row crops, with four passes. Weed control was adjusted for each tillage treatment. The number of weeding passes was adjusted according to the degree of weed infestation in each treatment.

All of the agricultural tools on wheat were 4 m wide. Wheel tracks were located on the same zones in 2021 as in previous years. However, as the tools used on maize and soybeans were 4.80 m wide, this, combined with harvesting operations, meant that the entire plot area might have been compacted by vehicle wheels over the last 16 years. An overview of the crop management system and sampling in 2021 is provided in Fig. 1. In 2021, winter wheat was irrigated before and after the soil sampling period (35 mm April 17–18, 30 mm on April 25–26, and 35 mm on June 7). Rainfall of 174 mm was recorded in May 2021, before the sampling period.

Soil structure

Visual assessment of soil structure

To understand where and why soil compaction occurs, soil structure was characterized morphologically from the observation face of a pit (1 m deep × 4 m wide), following 'soil profile' methodology (Boizard et al 2017; Peigné et al. 2013). In each plot of the experimental trial, one pit was dug perpendicular to the wheel tracks of soil cultivation machinery. The face of each pit was described macroscopically (on a vertical plane) in two steps. Three soil profiles per treatment (one per replicate) were performed at the flowering stage of winter wheat in May 2021.

First, we located all wheel tracks on the soil surface to indicate lateral stratification of the soil profile. In soil profiles, 3 types of lateral stratification are observed (See Fig. S1): the area of the profile located under the wheel tracks of field operations done after secondary tillage (visible at soil surface), called L1; the area of the profile located under the wheel tracks of secondary tillage operations, called L2; and part of the profile untouched by wheels since the primary tillage of the growing year, called L3. Then, soil layers delimited from the working depth of successive tillage tools used to prepare the seedbed for the current crop were located in the soil, indicating the vertical stratification of the soil profile. The intersection of vertical and horizontal stratifications naturally defines homogeneous soil compartments for our study (Fig. S1).

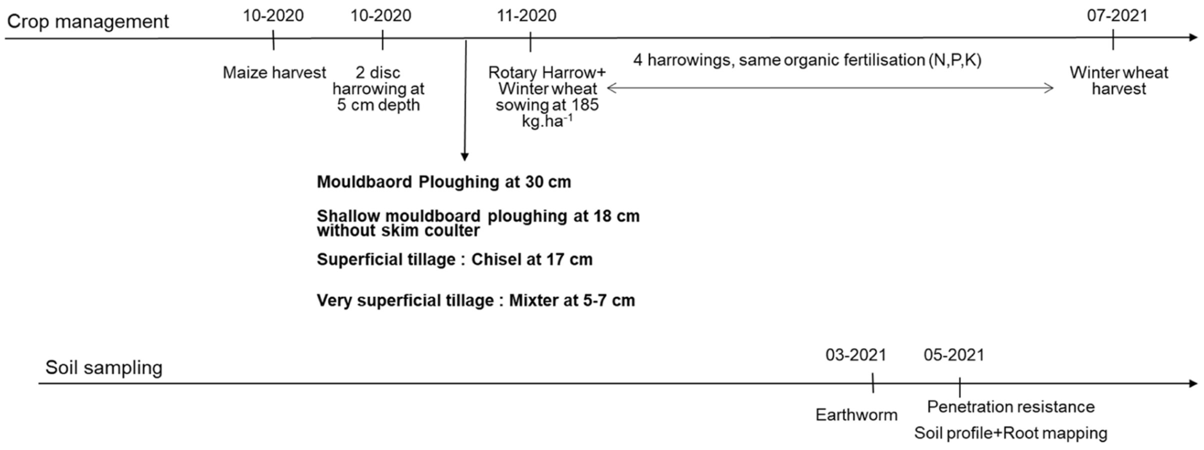


Fig. 1 Crop management and sampling dates. Organic fertilization (feather mill): 170 kg N/ha 10 kg P/ha, 10 kg K/ha

Secondly, we visually assessed the structural quality of every homogeneous compartment previously defined. We classified clods > 2 cm into three classes depending on the proportion of structural porosity visible: (1) delta (Δ) clods were clods with no visible structural porosity, with prominent edges and flat surfaces, evidence of severe compaction (Boizard et al. 2017); (2) Δb clods were moderately compacted clods with visible biological macropores (earthworms, roots) on their flat surfaces; and (3) gamma (Γ) clods were clods with a loose structure with clearly visible structural porosity. The clods had distinct physical characteristics. For instance, Δ clods had higher bulk density compared to Γ clods, which favor anoxic conditions (Curmi 1988) with lower biological activity (Vian et al. 2009). To assess structural quality visually, we observed the spatial distribution of the three types of clods in the homogeneous soil compartments (intersection of horizontal and vertical stratification). Then, we calculated the percentage of area occupied by each clod type in the soil (0–30 cm depth).

Soil penetration resistance

The soil penetration resistance was used to assess the state of the soil structure at different depths. It was measured in zones with no evidence of wheel marks (L3 zones; axis of the seeder). Soil penetration resistance was measured in May 2021 (Fig. 1) at the wheat flowering stage with a manual penetrometer "EIJKELKAMP". After choosing the most appropriate cone diameter (according to compaction level), the penetrometer was pushed vertically into the soil at an approximate constant rate of 2 cm per second for each handle. Soil resistance pressure was measured every 5 cm to 45 cm depth. The recorded resistance was standardized, taking into account the cross-section area of the cone. Penetration resistance was expressed in MPa. Ten randomly distributed measurements of soil penetration resistance were implemented per plot, only in areas with no recent wheel tracks. In total 30 measurements of soil penetration were obtained per tillage treatment (3 plots X 10 measurements). Gravimetric soil water content (% mass basis) was measured for every sub-replicate at five soil depths (0–5, 5–15, 15–20, 20–30, and 30–50 cm) to verify that soil moisture was similar between sampling zones. Moisture content averaged 14% over the 0–50 cm soil layer, varying from 14.5% in the first centimeters (0–5 cm) to 12.2% in the 30–50 cm soil layer.

Root growth and soil exploration

Root density

Roots were mapped by counting roots during soil profile observations. On the face of the soil profile, soil fragments were cut off with a knife to a thickness of 1 cm to refresh the face and highlight the roots. A grid (70 cm wide, 1 m long) was attached to the face of the soil profile (Pagès 1999; Pierret et al. 2007). The number of roots present in each cell ($2 * 2\ cm^2$) of the grid was counted and recorded, providing information on the number of roots present throughout the soil profile. Root density was calculated every 2 cm, from the soil surface to 70 cm soil depth, by summing the number of roots in the 35 cells of the 2-cm line of the grid.

Root density was counted twice for three soil profiles (*i.e.,* 6 measurements per treatment) at the flowering stage of winter wheat in May 2021. Grids were located on zones in the soil profile without wheel tracks (i.e., L3; Fig. 1).

Root biomass and morphological traits

Three cores of a depth of 45 cm were collected in each plot (i.e. 9 samples per treatment) in areas without recent wheel traffic (i.e., L3, same areas where penetrometry was measured). Each soil core with a diameter of 8 cm was divided into four sections to distinguish between the 0–5, 5–15, 15–30, and 30–45 cm soil layers. Soil samples were stored at -20 °C until further processing. Soil samples were soaked in buckets of water for 15 min. Then, the soil was washed off of the roots under running water over two sieves of 1 and 0.2 mm mesh-size (Freschet et al. 2021). After the soil was removed, all roots were collected from the sieves manually. Roots were then transferred and spread on a flat tray filled with water to remove any remaining impurities. Roots were not sorted between live and dead roots, and were all considered as absorptive roots ("fine roots") since root diameter was consistently < 1mm.

Sub-samples of roots were taken after washing to document root morphology measurements. Roots were cut and spread on a flat transparent tray to avoid any roots overlapping. They were then digitized at 600 dpi with a flatbed scanner (Epson V800 Pro) and analyzed using a digital image analysis system (RhizoVision Explorer, Seethepalli et al. 2021) to obtain root length and root diameter. Root dry mass was obtained after oven-drying scanned roots for 48 h at 60 °C. From these estimates, we calculated the average root diameter (RD, mm) and specific root length (SRL, cm. g^{-1}).

For each sample, the roots not used in the sub-sample were over dried for 48h at 60°C and weighed. The dry mass obtained was added to the one of sub-sample in order to get total root biomass. Root length density (cm. cm^{-3}) was obtained by multiplying root biomass in the soil core section by its estimated specific root length, and dividing it by the volume of the soil core section.

To assess root tip elongation, three undisturbed soil blocks were taken per tillage treatment (one per replicate). Each soil block measured 25 cm × 20 cm on the soil surface, centered on one crop row, and was 30 cm deep. These blocks were carefully washed and progressively opened to extract the whole root system. Six aliquots were collected that corresponded to entire branched roots from the soil surface to 30 cm depth. Each aliquot was divided into three sub-aliquots to distinguish 0–5, 5–15, and 15–30 cm rooting depth. Contrary to segmented roots scanned for assessing root diameter and length, the roots within each sub-aliquot were maintained intact. Each sub-aliquot was spread in a flat transparent tray, and was digitized with a flatbed scanner (Epson V800 Pro). Digital image analysis was used to estimate the number of root tips per root centimeter from the whole and branched roots that were scanned. Root tip is here defined as the root apical zone, from the apex to first lateral roots. Root endings created by cutting the roots during the division of sub-aliquots were identified and not considered as root tips. This provided a good overview of the importance of root tip elongation and spread of root branches.

Earthworm community and activity

Biomass, abundance, and density of earthworms

Earthworms were sampled in plots in areas without recent wheel traffic (i.e., L3, same areas where penetrometry was measured). In 2004, when the experiment was initiated, earthworm biomass was sampled using the formaldehyde method, and the results were published by Peigné et al. (2009). As this method presents a health risk to living organisms, and is not in accordance with organic farming principle, earthworm biomass was sampled using a hand sorting method after 2010 (Peigné et al. 2018). The earthworm population was sampled for the winter wheat crop in March 2021, three months before the wheat flowering stage (Fig. 1) when earthworms are very active. Six samples were assessed in each plot (i.e. 18 samples per treatment)., on a surface of 30 cm X 30 cm and to a depth of 30 cm. To sort earthworms, we simply dug the soil with a spade and worked fast enough to limit the number of anecic species that escaped. We poured the soil into a container, and then inspected the sides and bottom of the hole to detect any escaping earthworms. After carefully sorting the soil to capture all the earthworms in situ, we placed them in a bowl filled with fine soil. Jars containing the earthworms were placed in the shade (where it was cool) and were transported to the laboratory. There, the earthworms were cleaned and fixed with 4% of formaldehyde, classified by ecological categories (anecic, endogeic, and epigeic) and species. They were then counted and weighed (formaldehyde weight). Brown-headed anecic species (*Aporrectodea giardi*, *A. longa,* and *A. nocturna*) were grouped together because they occurred at relatively low abundances, it was not possible to separate the juveniles of each species, and they are assumed to have more similar functional roles compared to anecic *Lumbricus* species (Hoeffner et al. 2022).

Soil cores and earthworm burrow systems

Three cores (16 cm diameter, 25 cm depth) were collected in each plot (i.e. 9 samples per treatment) following the protocol described by Capowiez et al. (2021b, c) with 3 cores in each experimental plot. In sum, soil cores were manually collected using a PVC tube placed vertically on the soil. The soil around the tube was carefully excavated to a depth of a few centimeters using a small hand-spade. Then, only the soil just below the PVC tube was removed using a knife vertically at 1 cm intervals. After each centimeter, the tube was gently inserted into the soil using a hammer on top of the PVC tube. The process continued until the depth of the soil core in the PVC was 25 cm.

Then, the soil below the core was horizontally cut with a knife, and the core was put in a plastic bag. The macrofauna inside each core was killed to prevent further burrowing by adding 3 mL chloroform.

The macroporosity of the soil cores was analyzed by X-ray tomography using a medical scanner (BrightSpeed Exel 4, General Electric) at the INRAE Nancy Research Centre. The following settings were used: 50 kV, 130 mA, and 1.25 mm between images. The final image resolution was 0.38 mm per pixel. Images were first transformed into 8-bit images (using 1000 and 2000 HU as minimum and maximum grey-level values, respectively). The images were then binarized using a unique and fixed threshold for all cores, because the separation between peaks (void and soil matrix) in the grey-level histograms was easy.

Macroporosity was characterized using a variety of approaches. Volume was calculated as the sum of the volumes of burrows. The number of burrows larger and smaller than 2 cm^3 were differentiated. Estimated diameter was computed as the equivalent circular diameter for only the most circular 2D pores (i.e., for which circularity, computed in ImageJ, was higher than 0.8). Vertical barycenter was computed as the mean of the vertical center of mass of each macropore taking into account their respective volume. Vertical continuity was assessed by counting the number of burrows that had a vertical extension larger than 30% of the length of soil cores (i.e., 7.5 cm). The volume of macroporosity that was connected to the soil surface was quantified. All computations were done using ImageJ (Schindelin et al. 2012) and adapted macros.

Statistical analyses

The normal distribution and homoscedasticity of each variable was verified. The earthworm data did not follow a normal distribution, so a Kruskal Wallis test (non-parametric test) was performed (alpha = 0.05) followed by a Dunn test (alpha = 0.05) to perform pairwise comparisons if the Kruskal-Walis test is statically significant. For the same reason, analysis of the percentages of Δ, Δb and Γ clods observed in the soil profiles was also performed with a Kruskal Wallis test followed by a Dunn test. The composition of earthworm communities was analyzed using a Linear Discriminant Analysis (i.e. a projection of the data so that the differences between treatments are maximized). Data for burrow systems were analyzed with ANOVA (after log-transformation when required). The characteristics of the burrow systems were also analyzed using Linear Discriminant Analysis.

To analyze root density, biomass and traits of roots, along with penetration resistance, two statistical analyses were performed. First, linear mixed models with autoregressive errors were adjusted to account for the dependence of values with respect to soil depth. As fixed effects, the interaction of tillage treatment with soil depth used a polynomial function of 4 degrees (penetration resistance model) or 2 degrees (root trait models, root density models). An autoregressive (order 1 covariance) structure using soil depth was used. The random effects corresponded to the number of replicates. Post-hoc analysis were performed with tukey's multiple comparison test (alpha = 0.05). All statistical analyses were performed with R software (R Core Team 2016).

Results

Changes in soil compaction and root exploration across the soil profile

In the 0–45 cm soil layer, penetration resistance, root density, average diameter and specific root length were all significantly influenced by tillage practices in interaction with soil depth (Table 1). Root biomass was only affected by soil depth (Table 1).

The topsoil (0–15 cm) was characterized by the highest root biomass, regardless of the tillage treatment (Fig. 2a). All root traits were also similar with the exception of root density which was high with VST in the first 10 cm but lower compared to the other treatment if integrated on the depth range of 0–20 cm (Fig. 3). Significant differences in penetration resistance among the four tillage treatments were recorded in the surface 10 cm, with higher resistance for MP compared to ST and VST (Fig. 4). This result was explained by the presence of smoothing at 10 cm during the passage of the rotary harrow at sowing, which smoothed the soil deeply in plots that were very crumbled by ploughing.

Table 1 *P*-values (*** : < 0.001; ** : < 0.01; * : < 0.05) obtained from the different models testing the influence of tillage treatments and soil depth on soil penetration resistance, root density, biomass, average diameter, specific root length and root tip elongation

	Soil penetration resistance	Root density	Root biomass	Root average diameter	Specific root length	Root tip elongation
Depth	< 0.0001(***)	< 0.0001(***)	< 0.0001(***)	< 0.0001(***)	< 0.0001(***)	< 0.0001(***)
Tillage Treatment	0.9712	0.6305	0.6864	0.0015(**)	0.0006(***)	0.0738
Depth*Tillage Treatment	< 0.0001(***)	0.0097(**)	0.2489	0.0004(***)	0.0387(*)	0.0003(***)

Below the top soil (15–30 cm), root biomass decreased for all situations (Fig. 2a). Root average diameter was higher when no tillage occurred at this depth (ST, VST; Fig. 2b). In the case of VST, specific root length was lower, as well as root tip elongation (higher number of tips per root cm; Fig. 2c, d) and root density (Fig. 3). ST and VST also had higher resistance compared to the tilled situations at 20 cm deep (MP and SMP), with VST having the highest resistance (Fig. 4). At 30 cm depth, MP treatment was the only treatment being tilled at this depth, and remained with lower resistance values compared to the other treatments (SMP, ST, VST).

Higher soil compaction with less tillage was also strengthened by the qualitative observations of clods in the soil profiles. The main difference between soil profiles was the percentage of porous clods (Γ) being higher in MP (61%) compared to VST (29%; Fig. 5). In comparison, SMP and ST had intermediate percentages (40 and 41%, respectively).

Below 30 cm deep, no tillage operations were performed for any plots. Root biomass was the lowest and root traits were similar for all situations (Fig. 6a, b, c). However, VST exhibited lower resistance to penetration at 40 cm deep compared to MP (Fig. 4), and showed a tendency (not significant) to have higher root density compared to the more intensive tillage treatments (MP and SMP; Fig. 3).

Earthworm community and activity

Ten species of earthworms were identified, including one epigeic species, four anecic species with three brown-headed species, and six endogeic species with one unspecified endogeic species (Table S1). The communities were different between MP and SMP on one hand and ST on the other hand (Figure S2), with the first group characterized by a higher contribution of *A. icterica* and ST characterized by a higher contribution of *A. antipai* (Table S1). Our results showed that earthworm biomass was higher for VST (67.7 g.m^{-2}) compared to MP (34.9 g.m^{-2}) and SMP (38 g.m^{-2}) (Table 3, Kruskal Wallis p-value < 0.001), which was consistent with more compacted clods with visible earthworm macropores (Δb) found under VST (46%) compared to MP (22%) (Fig. 5). The difference in earthworm biomass for VST was attributed to greater anecic biomass (Kruskal Wallis p-value < 0.001) and total adult biomass (Kruskal Wallis p-value < 0.01). The only significant difference observed for abundance was the density of total adults, with significantly higher values in VST (26.5 individuals.m^{-2}) compared to MP (13.6 individuals.m^{-2}) and SMP (11.7 individuals.m^{-2}) (Kruskal Wallis p-value < 0.05) (Table 2).

Some representative burrow systems are presented in Fig. 6, showing many tubular macropores, assumed to be earthworm burrows, that were mainly vertically-oriented. Significant difference was observed for the total volume of burrows between SMP on one side and MP and ST on the other side, while VST hag intermediate values. No significant difference was revealed for diameter, or continuity (Table 3). The vertical barycenter was significantly higher for the two treatments MP and SMP, compared to ST and VST treatments which had shallower tillage and no soil inversion. SMP was characterized by a higher number of macropores (below and above 2 cm^3) compared to VST, ST, and MP, but a greater volume of burrows was connected to the surface under VST and ST treatments. Linear Discriminant Analysis (Fig. S2) showed that our tillage depth gradient is mainly separating VST and ST from MP plots along the first axis, and from SMP plots along the second axis. Situations with reduced tillage and no soil inversion (VST and ST) were mostly alike and were related to higher burrow diameter and higher volume of burrow connected to the surface.

Discussion

Soil stratification and compaction with long-term tillage reduction

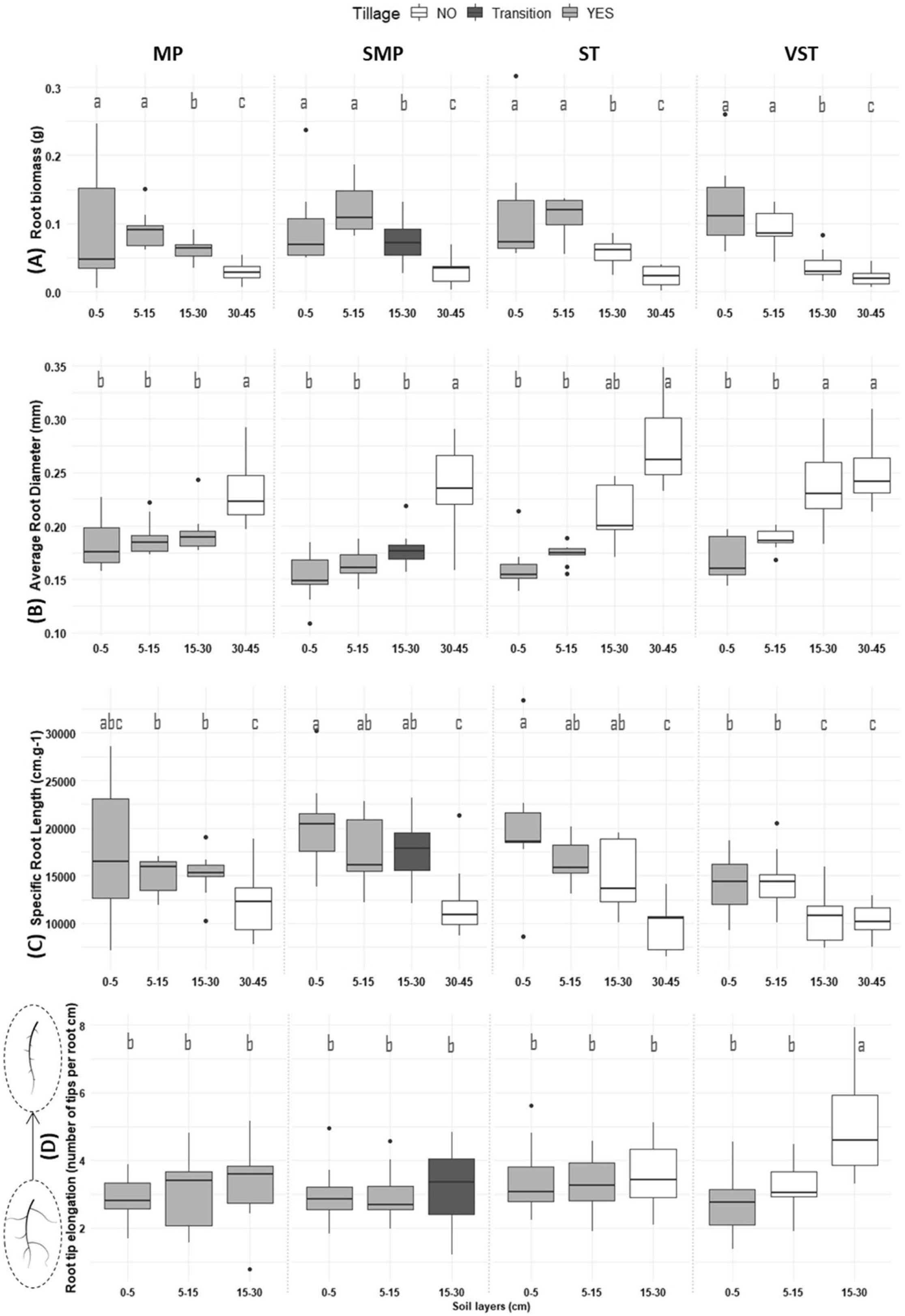
Reichert et al. (2016) suggested a conceptual framework on the evolution of soil structure under no-till with 4 phases. In the first three phases, after 1.5 years (initial phase), 3.5 years (intermediate), and 5 to 14 years (transitional phase), respectively, soil structure gradually improved due to its re-aggregation through biological activities in the 0–15 cm soil layer, before reaching the fourth phase (after 14 years) where the whole cultivated soil layer becomes stabilized and homogenous through the complete restoration of biological activity. While the transitional phase was indeed observed in our experiment after 10 years (Peigné et al. 2018), the final and complete restoration stage was not yet observed after 16 years. In our context, with a sensitive sandy loam soil, the stratification of the soil structure remained.

For the topsoil (0–15 cm) where tillage disturbance is involved for the whole soil layer in all treatments but VST, the most intensive tillage situation (MP) caused soil compaction at a depth of 10 cm due to the smoothing effect of the rotary harrow at sowing. It was not observed as a factor preventing root development. In the contrary, similar root biomass was observed across tillage treatments, and root density was slightly lower under VST at 20 cm, with more concentrate root density near the soil surface (Fig. 3).

Deeper, between 15 and 30 cm, non-ploughed treatments (ST and VST) had more compacted soil with higher penetration resistance and less porous clods (Γ). Root biomass was not significantly altered, although reduced root morphological traits changes suggest mechanical stress from compaction, impeding resource uptake and use efficiency. Root average diameter was found to be particularly linked to the tillage gradient though higher diameter when less tillage (Fig. 2b). Differences in root diameter for a given root order are associated with specific adaptations to environmental conditions (Ryser 1998). Morphological changes occur as a result of mechanical stress, with the thickening of root diameter representing one well-identified consequences in literature (Atwell 1990; Colombi et al. 2017; Schmidt et al. 2013), although it remains unclear whether these changes are the consequence of mechanical stress or an adaptation that favors root penetration capacity (Freschet et al. 2021).

Furthermore, lower root density, tip elongation, and specific root length were found under VST between 15 and 30 cm. These results underlined again the limitation effect of compacted soil layers on root cell elongation, and showed that the less tilled treatment (VST) was impacted the most in terms of root growth. Specific root length responses to resource limitation noticeably differ across publications (Freschet et al. 2018); however, this trait represents a key feature for evaluating the efficiency of resource acquisition by roots, where high specific root length indicates large absorptive surface with low cell mass, i.e. low structure and development costs. Furthermore, decrease in root tip elongation could impact root function in various ways. The root tip is a hotspot of soil-root exchange, through exudation, respiration, and water-nutrients uptake processes (Bidel et al. 2000; Laporte et al. 2013; Nguyen 2009).

As a consequence of compaction increase and root growth limitations, the reduction of tillage could also lead to a stratification of the root-soil exchanges, where most flux exchanges are concentrated in the first centimeters of soil. The deeper soil layers could then be mainly providing extra water pools and being less favorable for nutrient acquisition (nutrients mineralization decrease and immobilization increase). This pattern is supported in many no-till studies, with nutrients content and porosity improved in the first centimeters of no-till soil, while deeper centimeters are more compacted and less furnished with nutrients (Martínez et al. 2016; Krauss et al. 2018). Thus, tillage is a major factor shaping soil features and influencing soil-root interactions. In the past decades, productive grain systems were helped by ploughing operations because it temporarily creates a significant soil depth (20–30 cm deep) that allows fine roots to grow rapidly and homogeneously, and fast growing cultivars were improved simultaneously and can quickly acquire mineralized nutrients (Isaac et al. 2021; Roucou


Tillage
NO
Transition
YES
MP
SMP
ST
VST
(A) Root biomass (g)
0.0
0.1
0.2
0.3
(B) Average Root Diameter (mm)
0.10
0.15
0.20
0.25
0.30
0.35
(C) Specific Root Length (cm.g-1)
10000
15000
20000
25000
30000
(D) Root tip elongation (number of tips per root cm)
2
4
6
8
0-5
5-15
15-30
30-45
Soil layers (cm)
a a b c
a a b c
a a b c
a a b c
b b b a
b b b a
b b ab a
b b a a
abc b b c
a ab ab c
a ab ab c
b b c c
b b b
b b b
b b b
b b a

◂**Fig. 2** **a** Root biomass, **b** average root diameter, **c** specific root length and **d** root tip elongation (number of root tips per root cm) depending on tillage treatments (MP = moldboard ploughing; SMP = shallow moldboard ploughing; ST = superficial tillage, VST = very superficial tillage) and soil layers. For (**d**) root tip elongation, higher values mean less tip elongation. Lowercase letters indicate statistical group differences between boxplots (tuckey's multiple comparison, alpha = 0.05). White boxplots ("NO") indicate situations with no mechanical disturbance from tillage practices, light grey boxplots ("YES") indicate situations where mechanical disturbance is applied by tillage practices, and dark grey boxplots ("Transition") indicates situations where mechanical disturbance does not concern the whole sample, but only the upper part. This case is only found for the SMP treatment, where ploughing is done until 18–20 cm deep

et al. 2018). The development of cropping systems with less tillage might require new research on crops and cultivars with relevant root trait adaptations.

Earthworm community and activity improvements with long-term tillage reduction

The earthworm community was different between the two ploughed treatments (MP and SMP) and the ST treatment. However, the biomass of earthworms was higher in VST compared to MP and SMP, which was attributed to the higher body mass of adults and anecic earthworms. Those

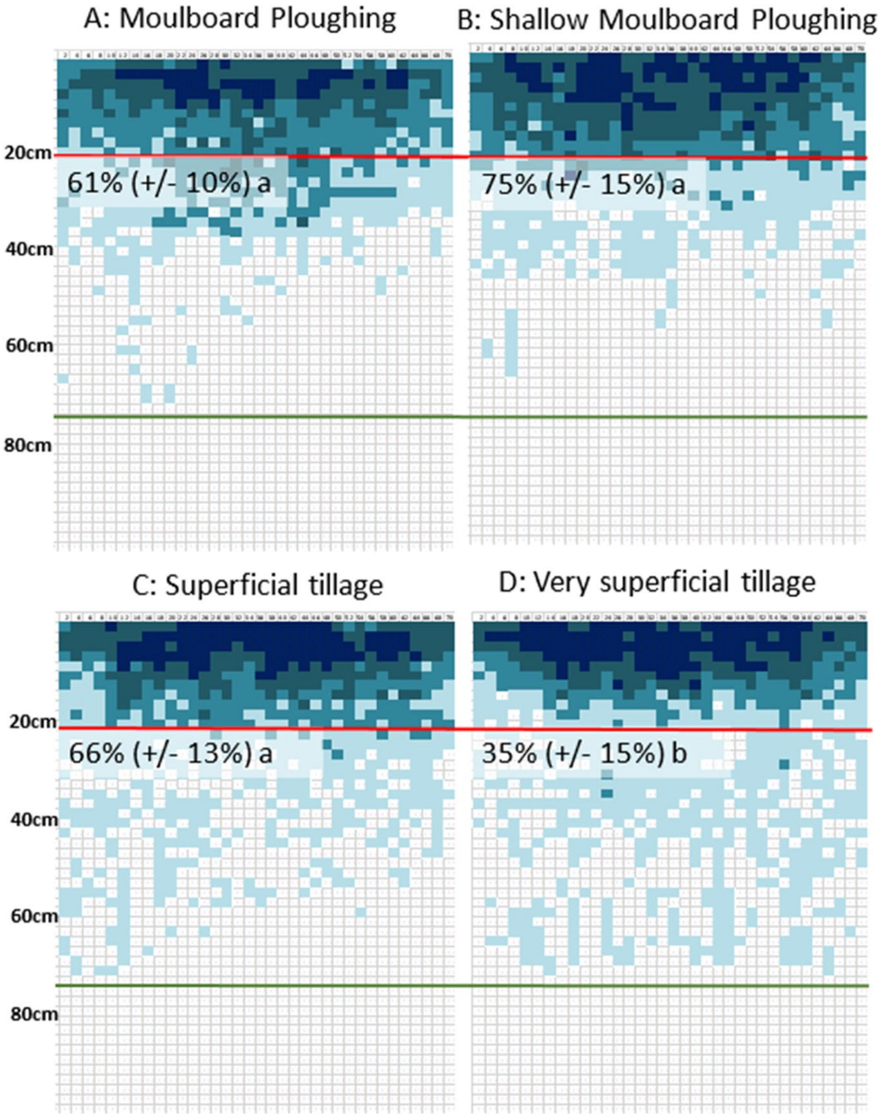


Fig. 3 **A**-**B**-**C**-**D**: Winter wheat root (mean values) maps obtained in moldboard ploughing (**A**), Shallow Moldboard Ploughing (**B**), Superficial Tillage (**C**) and Very Superficial Tillage (**D**) treatments – at the flowering stage of winter wheat in 2021. From light blue (1 root) to dark blue (more than 4 roots). Six individual values are used to calculate mean percentage (given above). Red lines indicate the depth of 20 cm where interaction between tillage treatment and depth was significant (*p*-value < 0.0125). Lowercase letters indicate statistical group differences considering the percentage of cells between 0 and 20 cm with at least one root observation. No statistical differences were found at deeper depths. Green line the end of the rooting at 72 cm depth

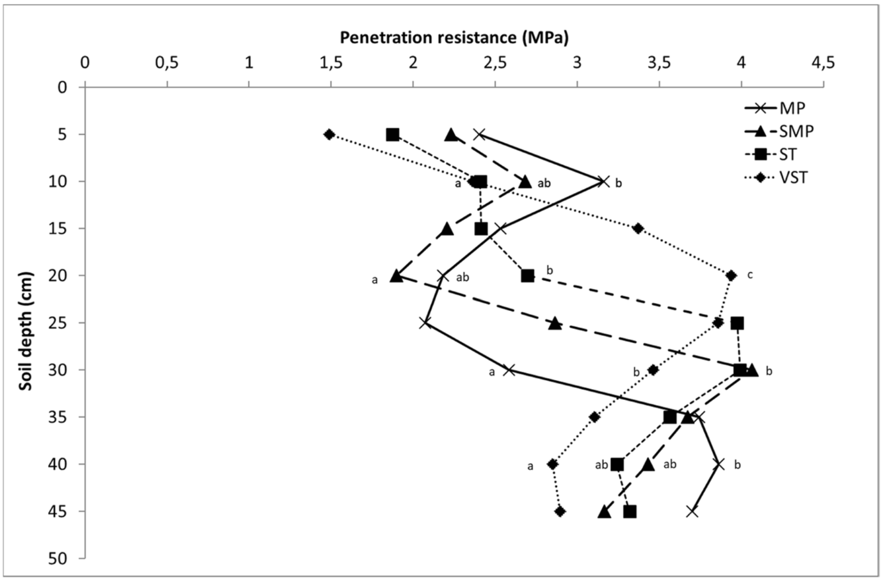


Fig. 4 Penetration resistance in MPa of the 4 tillage treatments from 5 to 45 cm depth in May 2021 under zones without wheel tracks. Thirty individual values are used to calculate the mean. At 10, 20, 30 and 40 cm different lowercase letters indicate statistical group differences according to tuckey's multiple comparison test (alpha = 0.05). MP: Moldboard ploughing; SMP: shallow moldboard ploughing; ST: superficial tillage; VST: very superficial tillage

are known for being more prevalent in lightly tilled soils (Pelosi et al. 2016) and our results are consistent with having slightly more Δb clods under VST (Fig. 5). These results support those obtained in other organic (Krauss et al. 2022) and conventional (Pelosi et al. 2014) cropping trials.. Low soil disturbance allows the persistence of anecic earthworms, as their vertical galleries are very sensitive to the destructive actions of soil inversion. These galleries are real burrows

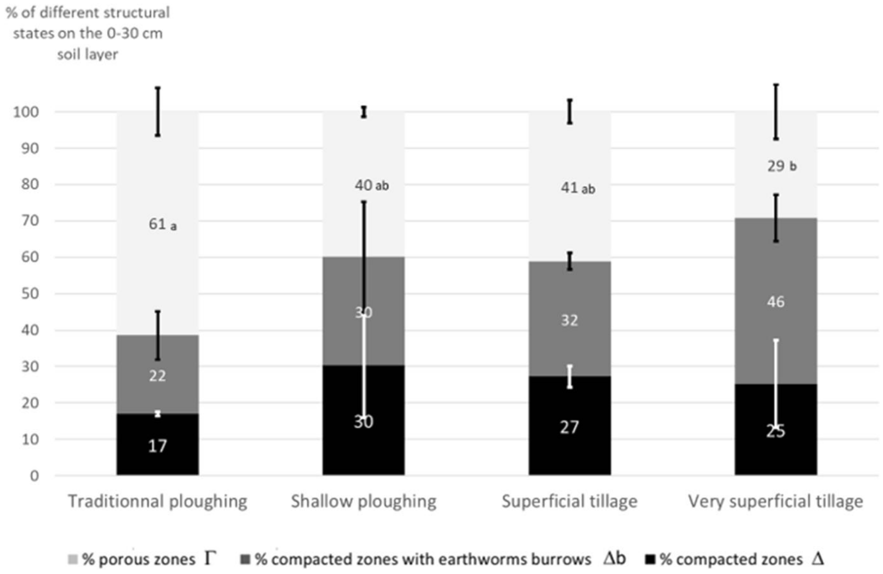


Fig. 5 Percentage of porous zones (Γ), compacted zones with earthworm burrows (Δb) and compacted zones (Δ) of the 4 tillage treatments observed in May 2021 in a soil profile (on the 0–30 cm soil layer in zones without wheel track). Three individual values are used to calculate the mean and standard error. MP: Moldboard ploughing; SMP: Shallow moldboard ploughing; ST: superficial tillage; VSP: very superficial tillage. No significant difference was found with the Kruskal-Wallis test (alpha = 0.05) for compacted zones (Δ) (*p*.value = 0,4), nor for compacted zones with earthworm burrows (Δb) (*p*.value = 0,07). Significant differences were found with the Kruskal-Wallis test (alpha = 0.05) for porous zones (Γ) (*p*.value = 0.025), and lowercase letters indicate statistical group differences (post-hoc Dunn's test) between tillage treatments

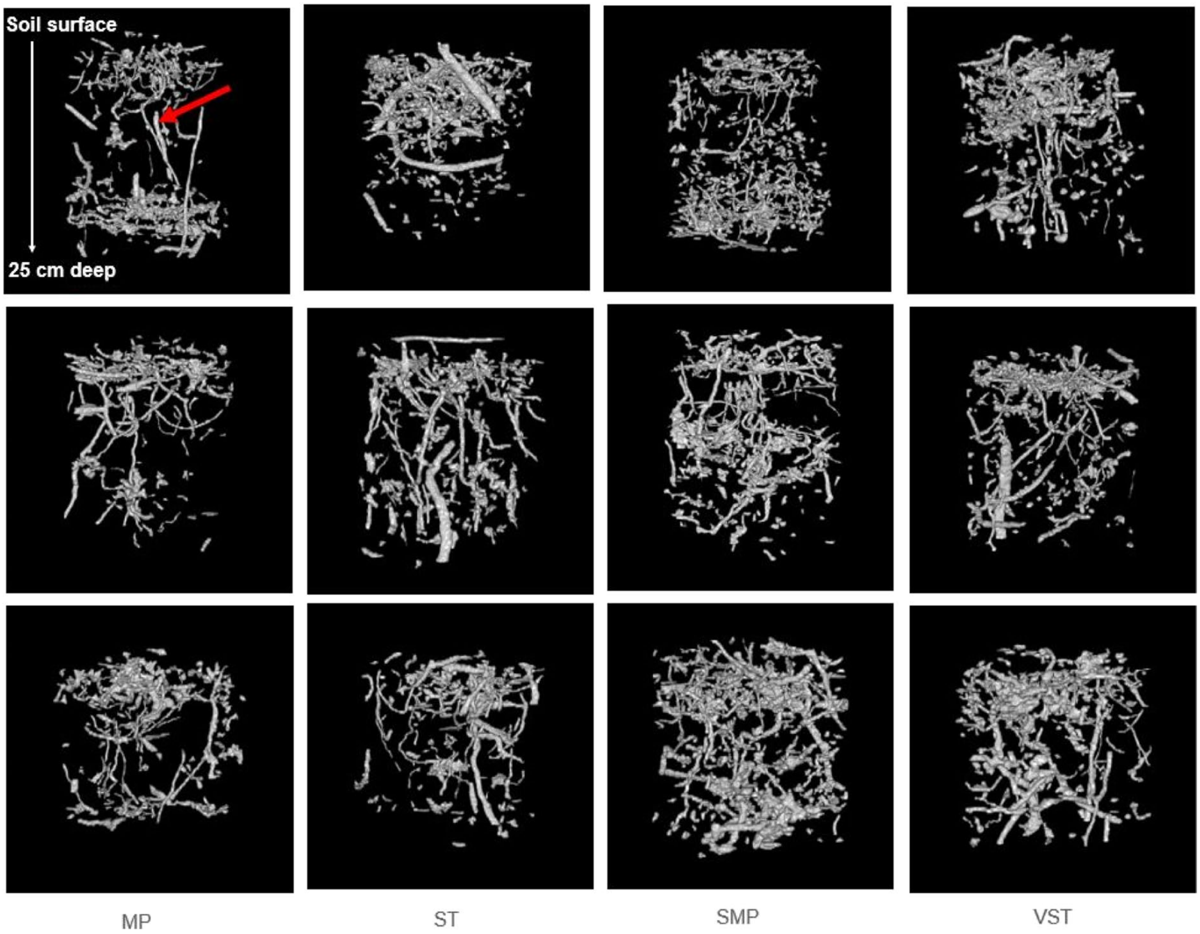


Fig. 6 Examples of burrows systems per plots and treatments. The red arrow indicates an example of tubular macropore. MP: Moldboard ploughing; SMP: Shallow moldboard ploughing; ST: superficial tillage; VSP: very superficial tillage)

that contribute to the survival and reproduction of these earthworms. However, the biomass recorded in our study (34.9 g.m^{-2} (MP) to 67.0 g.m^{-2} (VST)) was lower than that recorded in other experiments in France (Pelosi et al. 2014). This difference might be explained by the sandy soil and intensive organic cropping system at our site. Spring crops were intensively weeded, with low vegetation cover during crop rotation. The biomass measured in 2021 was similar to that in 2015, indicating that the population did not grow over the six years (Peigné et al. 2018). While this is purely speculative, we can imagine that more favorable conditions for earthworm activity improve the effect of reduced tillage with a stronger biological leverage to recreate functional soil porosity.

Earthworm biomass (mainly due to anecic worms) was higher in SMP compared to the other treatments in 2015. SMP might have presented the best compromise at that time to preserve earthworm biomass, with lower soil disturbance than traditional ploughing, but with less soil compaction than the non-ploughed treatments (27% of Δclods with SMP compared to 45% and 49% for ST and VST respectively in 2015, Peigné et al. 2018). However in 2021, compaction in the non-ploughed treatments was half that of 2015 in 2021 (in % of Δclods, same methodology). This compaction decrease is likely an effect of the duration of the experiment, gradually allowing earthworms

Table 2 Earthworm biomass (BM.g^{-2}) and abundance (Ind.m^{-2}) in March 2021 ($n=18$) per main ecological category. Data per species are in supplementary information. A,B; a,b corresponds to significant difference between treatments with the Kruskal-Wallis test ($\alpha=0.05$) followed by post-Hoc Dunn's ($\alpha=0.05$)

Species/adult or juvenil	Moldboard ploughing		Shallow moldboard ploughing		Superficial tillage		Very superficial tillage		Test Kruskal.Wallis: *p*.values	
	Ind.m^{-2}	BM g.m^{-2}	Ind.m^{-2}	BM g.m^{-2}	Ind.m^{-2}	BM g.m^{-2}	Ind.m^{-2}	BM g.m^{-2}	Ind.m^{-2}	BM g.m^{-2}
Total epigeic	4.9	1.3	0.6	0.1	3.1	1.4	3.1	1.1	0.368	0.322
Total epigeic adults	0.6	0.4	0.0	0.0	1.9	0.9	0.6	0.5	0.543	0.563
Total anecic	44.4	13.9a	43.2	18.7a	53.7	29.8ab	56.8	45.1b	0.231	> 0.001
Total anecic adults	1.2	2.4	1.9	3.9	3.7	5.8	7.4	15.2	0.256	0.119
Total endogeic	72.2	17.6	70.4	17.4	65.4	20.4	59.3	20.9	0.526	0.737
Total endogeic adults	10.5	6.8	9.3	7.2	15.4	6.9	17.9	10.6	0.122	0.293
Total	125.9	34.9a	122.2	38.0a	122.8	52.6ab	120.4	67.7b	0.967	> 0.001
Total adult	13.6A	9.1a	11.7A	10.4a	21.6AB	14.1a	26.5B	27.8b	0.011	0.008

to evolve and penetrate the compacted areas, as shown by the better proportion of clods containing visible earthworms macropores (Δb clods) under ST and VST in this study compared to the previous one, 6 years ago (Peigné et al. 2018) This gradual decrease in the negative effect of compaction can explain why the earthworms biomass was finally higher under VST treatment in 2021 compared to the ploughed treatments.

However, tomography observations of the burrow systems in 2021 were limited to the 0–20 cm depth, a layer where no clear differences were observed regarding soil compaction. Nevertheless, more burrows were connected to the soil surface in the non-ploughed treatments (ST and VST). This may be partly due to the increase of anecic earthworms activity (including epi-anecic ones), as VST was the only treatment that statistically indicated such anecic abundance increase. As these earthworms mainly feed on fresh organic matter at the soil surface, they had vertical burrows that are directly connected to the surface in order to access litter for their daily consumption (Capowiez et al. 2015). The connection of the macropores to the surface of the soil can support major positive functional change such as water infiltration and retention (Hangen et al. 2002; Shipitalo et al. 1990). However, we did not observe a larger total volume of pores nor galleries with larger diameters in non-ploughed treatments, linked to the higher presence of anecic earthworms and adults. The overall low earthworm biomass, regardless of treatment, prevented us from detecting any differences. Moreover, although the galleries of anecic earthworms are larger, they are generally less numerous in the soil, especially for epi-anecic earthworms compared to other earthworm categories (Capowiez et al. 2021a, b, c). Another reason for this lack of difference might be linked to the higher soil penetration resistance below 15 cm in the non-ploughed treatments. Soil bulk density negatively affects the burrowing behavior of earthworms (Capowiez et al. 2021a, b, c; Rushton 1986). Thus, after 16 years of very superficial tillage, the activity of earthworms likely failed to increase soil macroporosity compared to ploughed soils in our pedoclimatic and agronomic contexts.

Finally, the greater abundance of anecic earthworms might explain the decrease in penetration resistance at greater depth (40 cm) under VST

Table 3 Main characteristics of the burrow systems observed in the sampled soil cores (diameter = 16 cm and depth = 25 cm

3D burrow characteristics	Moldboard ploughing	Shallow moldboard ploughing	Superficial tillage	Very superficial tillage	Anova test: *p*.values
Volume of burrows (cm^3)	28.85b	55.89a	36.29ab	46.11ab	0.01
Diameter (mm)	7.18	7.33	7.50	7.56	0.13
Vertical barycenter (cm)	77.61a	88.60a	45.13b	53.45b	< 0.001
Continuity#	5.88	4.67	6.00	6.12	0.59
Number of 2D macropores whose area was lower than 2 cm^3	120.2b	227.3a	157.2b	159.3b	< 0.001
Number of 2D macropores whose area was larger than 2 cm^3	50.89b	84.8a	50.44b	59.89ab	0.0064
Macroporosity connected to the surface (cm^3)	4.90b	8.61b	11.81a	14.78a	0.004

since these earthworms are the only ones to burrow so deep (Capowiez et al. 2012). Unfortunately, no information regarding burrow systems was available due to the difficulty in sampling soil cores at these depths. Anecic and epi-anecic earthworms build deep vertical burrows that improve macroporosity, potentially enhancing root growth at depth. Capowiez et al. (2009) showed the presence of larger macropores in reduced tillage compared to ploughing at 30–40 cm depth in loamy soils, supporting this possible improvement in depth. Other studies also identified this beneficial role of deep vertical burrows made by anecic earthworm species or roots, and their associations with greater oxygenation and water infiltration at depth (Wendel et al. 2022).

Conclusions

After 16 years of organic conservation tillage in a sandy loam soil, our study suggests that the first centimeters of soil are crucial to observe the main benefits of CT practices in terms of soil functioning. Without ploughing (ST, VST), better connection of the galleries to the soil surface are key features to sustain water infiltration and nutrients concentration (Peigné et al. 2018). But in spite of increased earthworm's abundance, the absence of ploughing was still associated with a substantial stratification of soil and root features, i.e. differences between soil layers. Notably under VST and below 15 cm, the soil became denser, and altered the morphological traits of roots, with higher root diameter and lower specific root suggesting less efficient resource uptake by roots. Further analysis on microorganism activities and nutrients fluxes should be performed to determine the nature and magnitude of plant-soil interaction changes.

Our results raise questions on current recommendations for tillage techniques in such sensitive sandy loam soil. In sandy soils, intermittent or strategic tillage with shallow mouldboard ploughing might avoid compaction problems, which are, ultimately, poorly compensated for by biological activity in the long term under conservation tillage. Of importance, a diversified but relatively short and intensive, crop rotation with only temporary cover crops was used in this study. Our study is therefore strengthening that soil health goals cannot be targeted solely through the reduction of tillage and the minimization of soil disturbance. Within an integrated approach, all other soil health principles must be considered too (soil armor, plant diversity, living roots, and livestock integration; Natural Resources Conservation Service, USDA, n.d.). New and more disruptive practices such as temporary grasslands, agroforestry, or permanent cover crops, could be integrated in no-till systems to sustain soil health and functions, meet current expectations about "ecological intensification".

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors: Olivier Duchene and Adeline Cadiergues for roots study, Yvan Capowiez and

Vincent Ducasse for earthworm study, Jean-François Vian, Olivier Duchene, Thomas Lhuillery and Joséphine Peigné for soil physical study. The first draft of the manuscript was written by Olivier Duchene and Joséphine Peigné, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

References

Atwell BJ (1990) The effect of soil compaction on wheat during early tillering. I. Growth, development and root structure. New Phytol 115:29–35. https://doi.org/10.1111/j.1469-8137.1990.tb00918.x

Bastardie F, Capowiez Y, Cluzeau D (2005) 3D characterisation of earthworm burrow systems in natural soil cores collected from a 12-year-old pasture. Appl Soil Ecol 30(1):34–46. https://doi.org/10.1016/j.apsoil.2005.01.001

Bidel LPR, Renault P, Pages L, Riviere LM (2000) Mapping meristem respiration of *Prunus persica* (L.) Batsch roots: potential respiration of the meristems, O2 diffusional constraints, and combined effects on root growth. J Exp Bot 51:755–768. https://doi.org/10.1093/jxb/51.345.755

Bohoussou YN, Kou YH, Yu WB, Lin B, Virk AL, Zhao X, Dang YP, Zhang HL (2022) Impacts of the components of conservation agriculture on soil organic carbon and total nitrogen storage: A global meta-analysis. Sci Tot Environ 842:156822. https://doi.org/10.1016/J.SCITOTENV.2022.156822

Boizard H, Peigné J, Sasal MC, de Fátima Guimarães M, Piron D, Tomis V, Vian JF, Cadoux S, Ralisch R, Tavares Filho J, Heddadj D, de Battista J, Duparque A, Franchini JC, Roger-Estrade J (2017) Developments in the “profil cultural” method for an improved assessment of soil structure under no-till. Soil Tillage Res 173:92–103. https://doi.org/10.1016/j.still.2016.07.007

Bottinelli N, Jouquet P, Capowiez Y, Podwojewski P, Grimaldi M, Peng X (2015) Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? Soil Tillage Res 146:118–124. https://doi.org/10.1016/j.still.2014.01.007

Casagrande M, Peigné J, Payet V, Mäder P, Sans FX, Blanco-Moreno JM, Antichi D, Bàrberi P, Beeckman A, Bigongiali F, Cooper J, Dierauer H, Gascoyne K, Grosse M, Heß J, Kranzler A, Luik A, Peetsmann E, Surböck A, …, David C (2015) Organic farmers’ motivations and challenges for adopting conservation agriculture in Europe. Org Agr 6:281–295. https://doi.org/10.1007/s13165-015-0136-0

Capowiez Y, Cadoux S, Bouchant P, Ruy S, Roger-Estrade J, Richard G, Boizard H (2009) The effect of tillage type and cropping system on earthworm communities, macroporosity and water infiltration. Soil Tillage Res 105(2):209–216. https://doi.org/10.1016/j.still.2009.09.002

Capowiez Y, Sammartino S, Cadoux S, Bouchant P, Richard G, Boizard H (2012) Role of earthworm in regenerating soil structure after compaction in reduced tillage systems. Soil Biol Biochem 55:93–103. https://doi.org/10.1016/j.soilbio.2012.06.013

Capowiez Y, Bottinelli N, Sammartino S, Michel E, Jouquet P (2015) Morphological and functional characterisation of the burrow systems of six earthworm species (Lumbricidae). Biol Fertil Soils 51:869–877. https://doi.org/10.1007/s00374-015-1036-x

Capowiez Y, Lévèque T, Pelosi C, Capowiez L, Mazzia C, Schreck E, Dumat C (2021a) Using the ecosystem engineer concept to test the functional effects of a decrease in earthworm abundance due to an historic metal pollution gradient. Appl Soil Ecol 158:103816. https://doi.org/10.1016/j.apsoil.2020.103816

Capowiez Y, Gilbert F, Valat A, Poggiale JC, Bonzom JM (2021b) Depth distribution of soil organic matter and burrowing activity of earthworms - mesocosm study using X-ray tomography and luminophores. Biol Fert Soils 57:337–346. https://doi.org/10.1007/s00374-020-01536-y

Capowiez Y, Sammartino S, Keller T, Bottinelli N (2021c) Decreased burrowing activity of endogeic earthworms and effects on water infiltration in response to an increase in soil bulk density. Pedobiologia 85–86:150728. https://doi.org/10.1016/j.pedobi.2021.150728

Chabert A, Sarthou JP (2020) Conservation agriculture as a promising trade-off between conventional and organic agriculture in bundling ecosystem services. Agr Ecosystem Environm 292:106815. https://doi.org/10.1016/j.agee.2019.106815

Colombi T, Kirchgessner N, Walter A, Keller T (2017) Root tip shape governs root elongation rate under increased soil strength. Plant Physiol 174:2289–2301. https://doi.org/10.1104/pp.17.00357

Cooper J, Baranski M, Stewart G, Nobel-de Lange M, Bàrberi P, Fließbach A, Peigné J, Berner A, Brock C, Casagrande M, Crowley O, David C, de Vliegher A, Döring TF, Dupont A, Entz M, Grosse M, Haase T, Halde C, …, Mäder P (2016) Shallow non-inversion tillage in organic farming maintains crop yields and increases soil C stocks: a meta-analysis. Agron Sustain Dev 36(1). https://doi.org/10.1007/s13593-016-0354-1

Curmi P (1988) Structure, espace poral du sol et fonctionnement hydrique. Analyse de quelques cas concrets. Sci Du Sol 26(3):203–214

Drewry JJ (2006) Natural recovery of soil physical properties from treading damage of pastoral soils in New Zealand and Australia: a review. Agr Ecosyst Environ 114:159–169. https://doi.org/10.1016/j.agee.2005.11.028

Freschet GT, Violle C, Bourget MY, Scherer-Lorenzen M, Fort F (2018) Allocation, morphology, physiology, architecture: the multiple facets of plant above- and below-ground responses to resource stress. New Phytol. 219:1338–1352. https://doi.org/10.1111/nph.15225

Freschet GT, Pagès L, Iversen CM, Comas LH, Rewald B, Roumet C, Klimešová J, Zadworny M, Poorter H, Postma JA, Adams TS, Bagniewska-Zadworna A, Bengough AG, Blancaflor EB, Brunner I, Cornelissen JHC, Garnier E, Gessler A, Hobbie SE, Meier IC, Mommer L, Picon-Cochard C, Rose L, Ryser P, Scherer-Lorenzen M, Soudzilovskaia NA, Stokes A, Sun T, Valverde-Barrantes OJ, Weemstra M, Weigelt A, Wurzburger N, York LM, Batterman SA, Gomes de Moraes M, Janeček Š, Lambers H, Salmon V, Tharayil N, McCormack ML (2021) A starting guide to root ecology: strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. New Phytol 232:973–1122. https://doi.org/10.1111/nph.17572

Hangen E, Buczko U, Bens O, Brunotte J, Hüttl RF (2002) Infiltration patterns into two soils under conventional and conservation tillage: influence of the spatial distribution of plant root structures and soil animal activity. Soil Tillage Res 63:181–186. https://doi.org/10.1016/S0167-1987(01)00234-3

Henneron L, Bernard L, Hedde M, Pelosi C, Villenave C, Chenu C, Bertrand M, Girardin C, Blanchart E (2015) Fourteen years of evidence for positive effects of conservation agriculture and organic farming on soil life. Agron Sustain Dev 35:169–181. https://doi.org/10.1007/s13593-014-0215-8

Hoeffner K, Butt KR, Monard C, Frazao J, Pérès G, Cluzeau D (2022) Two distinct ecological behaviours within anecic earthworm species in temperate climates. Eur J Soil Biol 113:103446

IFOAM (n.d.) The four principles of organic agriculture [WWW Document]. https://ifoam.bio/why-organic/shaping-agriculture/four-principles-organic (accessed 7.8.23)

Isaac ME, Nimmo V, Gaudin ACM, Leptin A, Schmidt JE, Kallenbach CM, Martin A, Entz M, Carkner M, Rajcan I, Boyle TD, Lu X (2021) Crop domestication, root trait syndromes, and soil nutrient acquisition in organic agroecosystems: a systematic review. Front Sustain Food Syst 5:317. https://doi.org/10.3389/fsufs.2021.716480

Kassam A, Friedrich T, Derpsch R (2022) Successful experiences and lessons from conservation agriculture worldwide. Agronomy 12(4). https://doi.org/10.3390/agronomy12040769

Kan ZR, Liu QY, He C, Jing ZH, Virk AL, Qi JY, Zhao X, Zhang HL (2020) Responses of grain yield and water use efficiency of winter wheat to tillage in the North China Plain. Field Crop Res 249:107760. https://doi.org/10.1016/j.fcr.2020.107760

Krauss M, Mäder P, Peigné J, Cooper J (2018) Conservation tillage in organic farming. In Köpke U (ed) Improving organic crop cultivation, 1st edn. Burleigh Dodds Science Publishing, pp 153–177

Krauss M, Berner A, Perrochet F, Frei R, Niggli U, Mäder P (2020) Enhanced soil quality with reduced tillage and solid manures in organic farming-a synthesis of 15 years. Sci Rep 10:4403. https://doi.org/10.1038/s41598-020-61320-8

Krauss M, Wiesmeier M, Don A, Cuperus F, Gattinger A, Gruber S, Haagsma WK, Peigné J, Chiodelli Palazzoli M, Schulz F, van der Heijden MGA, Vincent-Caboud L, Wittwer RA, Zikeli S, Steffens M (2022) Reduced tillage in organic farming affects soil organic carbon stocks in temperate Europe. Soil Tillage Res 216:105262. https://doi.org/10.1016/j.still.2021.105262

Kuntz M, Berner A, Gattinger A, Scholberg JM, Mäder P, Pfiffner L (2013) Influence of reduced tillage on earthworm and microbial communities under organic arable farming. Pedobiologia 56(4–6):251–260. https://doi.org/10.1016/j.pedobi.2013.08.005

Laporte MA, Denaix L, Pages L, Sterckeman T, Flenet F, Dauguet S, Nguyen C (2013) Longitudinal variation in cadmium influx in intact first order lateral roots of sunflower (*Helianthus annuus.* L). Plant Soil 372:581–595. https://doi.org/10.1007/s11104-013-1756-3

Martínez I, Chervet A, Weisskopf P, Sturny WG, Etana A, Stettler M, Forkman J, Keller T (2016) Two decades of no-till in the Oberacker long-term field experiment: Part I. Crop yield, soil organic carbon and nutrient distribution in the soil profile. Soil Tillage Res 163:141–151. https://doi.org/10.1016/j.still.2016.05.021

Natural Resources Conservation Service, USDA (n.d.) Soil health [WWW Document]. URL https://www.nrcs.usda.gov/conservation-basics/conservation-by-state/north-dakota/soil-health (accessed 7.8.23).

Nguyen C (2009) Rhizodeposition of Organic C by Plant: Mechanisms and Controls. In: Lichtfouse E, Navarrete M, Debaeke P, Véronique S, Alberola C (eds) Sustainable Agriculture. Springer, Dordrecht, pp 97–123. https://doi.org/10.1007/978-90-481-2666-8_9

Pagès L (1999) Root system architecture: from its representation to the study of its elaboration. Agronomie 19:295–304

Pierret A, Doussan C, Capowiez Y, Bastardie F, Pagès L (2007) Root Functional Architecture: A framework for modeling the Interplay between Roots and Soil. Vadose Zone J 6(2):269–281. https://doi.org/10.2136/vzj2006.0067

Peigné J, Ball BC, Roger-Estrade J, David C (2007) Is conservation tillage suitable for organic farming? A Review. Soil Use Manag 23(2):129–144. https://doi.org/10.1111/j.1475-2743.2006.00082.x

Peigné J, Cannavaciuolo M, Gautronneau Y, Aveline A, Giteau JL, Cluzeau D (2009) Earthworm populations under different tillage systems in organic farming. Soil Tillage Res 104(2):207–214. https://doi.org/10.1016/j.still.2009.02.011

Peigné J, Vian JF, Cannavacciuolo M, Lefevre V, Gautronneau Y, Boizard H (2013) Assessment of soil structure in the transition layer between topsoil and subsoil using the profil cultural method. Soil Tillage Res 127:13–25. https://doi.org/10.1016/j.still.2012.05.014

Peigné J, Vian JF, Payet V, Saby N (2018) Soil fertility after 10 years of conservation tillage in organic farming. Soil Tillage Res 175:194–204. https://doi.org/10.1016/j.still.2017.09.008

Pelosi C, Pey B, Hedde M, Caro G, Capowiez Y, Guernion M, Peigné J, Piron D, Bertrand M, Cluzeau D (2014) Reducing tillage in cultivated fields increases earthworm functional diversity. Appl Soil Ecol 83:79–87. https://doi.org/10.1016/j.apsoil.2013.10.005

Pelosi C, Pey B, Caro G, Cluzeau D, Peigné J, Bertrand M, Hedde M (2016) Dynamics of earthworm taxonomic and functional diversity in ploughed and no-tilled cropping systems. Soil Tillage Res 156:25–32. https://doi.org/10.1016/j.still.2015.07.016

R Core Team (2016) R: A language and environment for statistical computing. R foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Reichert JM, da Rosa VT, Vogelmann ES, da Rosa DP, Horn R, Reinert DJ, Sattler A, Denardin JE (2016) Conceptual framework for capacity and intensity physical soil properties affected by short and long-term (14 years) continuous no-tillage and controlled traffic. Soil Tillage Res 158:123–136. https://doi.org/10.1016/j.still.2015.11.010

Roucou A, Violle C, Fort F, Roumet P, Ecarnot M, Vile D (2018) Shifts in plant functional strategies over the course of wheat domestication. J Appl Ecol 55:25–37. https://doi.org/10.1111/1365-2664.13029

Rushton SP (1986) The effects of soil compaction on *Lumbricus terrestris* and its possible implications for populations on land reclaimed from open-cast coal mining. Pedobiologia 29:85–90

Ryser P (1998) Intra- and interspecific variation in root length, root turnover and the underlying parameters. In: Lambers H, Poorter H, Van Vuuren MMI (eds) Variation in plant growth. Backhuys Publishers, Leiden, Physiological mechanisms and ecological consequences, pp 441–465

Seethepalli A, Dhakal K, Griffiths M, Guo H, Freschet GT, York LM (2021) RhizoVision Explorer: open-source software for root image analysis and measurement standardization. AoB Plants 13(6):056

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Cardona A (2012) Fiji: an open-source platform for biological-image analysis. Nat Methods 9:676–682. https://doi.org/10.1038/nmeth.2019

Schmidt S, Gregory PJ, Grinev DV, Bengough AG (2013) Root elongation rate is correlated with the length of the bare root apex of maize and lupin roots despite contrasting responses of root growth to compact and dry soils. Plant Soil 372:609–618. https://doi.org/10.1007/s11104-013-1766-1

Shipitalo MJ, Edwards WM, Dick WA, Owens LB (1990) Initial storm effect on micropore transport of surface-applied chemicals in no-till soil. Soil Sci Soc Am J 54:1530–1536. https://doi.org/10.2136/sssaj1990.03615995005400060004x

Soane BD, Ball BC, Arvidsson J, Basch G, Moreno F, Roger-Estrade J (2012) No-till in northern, western and south-western Europe: A review of problems and opportunities for crop production and the environment. Soil Tillage Res 118:66–87. https://doi.org/10.1016/j.still.2011.10.015

Vian JF, Peigne J, Chaussod R, Roger-Estrade J (2009) Effects of four tillage systems on soil structure and soil microbial biomass in organic farming. Soil Use Manag 25(1):1–10. https://doi.org/10.1111/j.1475-2743.2008.00176.x

Wendel AS, Bauke SL, Amelung W, Knief C (2022) Root-rhizosphere-soil interactions in biopores. Plant Soil 475(1–2):253–277. https://doi.org/10.1007/s11104-022-05406-4

Young MD, Ros GH, de Vries W (2021) Impacts of agronomic measures on crop, soil, and environmental indicators: A review and synthesis of meta-analysis. Agr Ecosyst Environ 319:107551. https://doi.org/10.1016/j.agee.2021.107551